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(54) Title: METHOD TO DETECT BIOLOGICALLY ACTIVE, ALLERGEN-SPECIFIC IMMUNOGLOBULINS

(57) Abstract

The present invention includes a method to detect a biologically active, allergen-specific immunoglobulin using a Fc epsilon receptor (FcεR) molecule. Such a method can detect biologically active, allergen-specific immunoglobulins not detectable by a process using anti-IgE antibodies. The present invention also relates to kits to perform such methods. The present invention also includes a heat stable, biologically active, allergen-specific immunoglobulin.

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METHOD TO DETECT BIOLOGICALLY ACTIVE, ALLERGEN-SPECIFIC IMMUNOGLOBULINS

FIELD OF THE INVENTION

The present invention relates to a novel method to detect biologically active, allergen-specific immunoglobulins. The present invention also includes novel kits to detect such immunoglobulins. The present invention also includes novel immunoglobulins.

BACKGROUND OF THE INVENTION

Diagnosis of disease and determination of treatment efficacy are important tools in medicine. In particular, detection of immunoglobulin E (IgE) production in an animal can be indicative of disease. Such diseases include, for example, allergy, asthma, atopic disease, hyper IgE syndrome, internal parasite infections and B cell neoplasia. IgE-mediated allergic diseases, such as asthma, hay fever, atopic dermatitis and other skin diseases, affect up to 30 percent of the human population of industrialized countries. In addition, reduction of IgE production in an animal following a treatment is indicative of the efficacy of the treatment, such as when using treatments intended to disrupt IgE production.

Until recently, the diagnosis of IgE-mediated disease and the identification of the responsible allergens have been based primarily on a patient's history, on skin testing and on serological *in vitro* detection of IgE using antibodies that bind selectively to epsilon isotype antibodies (i.e., anti-IgE antibodies). These anti-IgE antibodies, however, have several drawbacks: many anti-IgE antibodies cross-react with other antibody isotypes, such as gamma isotype antibodies, leading to assays having low specificity; results from tests using anti-IgE antibodies often do not correlate with biological test results, such as skin tests, provocation tests, or a patient's history; anti-IgE antibodies can be inhibited from binding to IgE when the IgE is bound to its respective allergen; anti-IgE antibodies cannot distinguish between biologically active and inactive IgE; and anti-IgE antibodies cannot identify other IgE-like molecules that interact with the high affinity receptor for IgE to cause allergy.

The high affinity receptor for IgE ($Fc_\epsilon RI$) consists of three protein chains: alpha, beta and gamma. Prior investigators have disclosed the nucleic acid sequence for: the alpha chain (U.S. Patent No. 4,962,035, by Leder, issued October 9, 1990; U.S. Patent No. 5,639,660, by Kinet et al., issued June 17, 1997; Kochan et al., *Nucleic Acids Res.* 16:3584, 1988; Shimizu et al., *Proc. Natl. Acad. Sci. USA* 85:1907-1911, 1988; and Pang et al., *J. Immunol.* 151:6166-6174, 1993); the beta chain (Kuster et al., *J. Biol. Chem.* 267:12782-12787, 1992); and the gamma chain (Kuster et al., *J. Biol. Chem.* 265:6448-6452, 1990). These investigators, however, did not disclose the use of such a receptor, or subunits thereof, to detect IgE in animals, such as allergy-mediating IgE.

There have been a few reports of the use of the human $Fc_\epsilon RI$ alpha chain to detect total IgE in human sera, but to the inventors' knowledge, such reports do not present evidence of the ability to use the $Fc_\epsilon RI$ alpha chain to identify allergen-specific IgE; see, for example, Japanese Patent Application Publication 8-101194, by Ami, published April 16, 1996; and Suto et al, *Jpn. J. Dermatol. 106*, 1377-1384, 1996. Not demonstrating the ability of $Fc_\epsilon RI$ to identify allergen-specific IgE is relevant because there are teachings that binding of allergen to IgE inhibits the ability of the IgE to bind to the $Fc_\epsilon RI$ alpha chain and that, as such, $Fc_\epsilon RI$ might not be a good reagent for the detection of allergen-specific IgE ; see, for example, Stampfli et al, *Journal of Immunology 155*, 2948-2954, 1995.

Thus, methods and kits are needed in the art that will provide detection of biologically active, allergen-specific immunoglobulins.

SUMMARY OF THE INVENTION

The present invention includes a method to detect a biologically active immunoglobulin that selectively binds to a specific allergen in a mammal. The method includes the steps of: (a) contacting a putative biologically active, allergen-specific immunoglobulin-containing composition from the mammal with an isolated Fc_ϵ receptor ($Fc_\epsilon R$) molecule and with the specific allergen under conditions suitable for formation of a $Fc_\epsilon R$:immunoglobulin:allergen complex; and (b) determining the presence of the immunoglobulin by detecting the complex, the presence of the complex indicating the presence of the immunoglobulin.

One embodiment of the present invention is a method to detect a biologically active, allergen-specific immunoglobulin in a mammal, wherein a process using anti-IgE antibodies does not detect the immunoglobulin. The method includes the steps of: (a) contacting a putative biologically active, allergen-specific immunoglobulin-containing composition from the mammal with an isolated mammalian $Fc_\epsilon R$ molecule and with the specific allergen under conditions suitable for formation of a $Fc_\epsilon R$:immunoglobulin:allergen complex; and (b) determining the presence of the immunoglobulin by detecting the complex, the presence of the complex indicating the presence of the immunoglobulin.

The present invention also includes a kit for detecting a biologically active, allergen-specific immunoglobulin in a composition. The kit includes a mammalian $Fc_\epsilon R$ molecule, the specific allergen, and a means for detecting the immunoglobulin.

In one embodiment, a biologically active, allergen-specific immunoglobulin of the present invention is heat stable. The present invention also includes an isolated biologically active, allergen-specific immunoglobulin that is heat stable and that selectively binds to a mammalian $Fc_\epsilon R$ molecule.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows a comparison of results obtained from testing a chronic rhinitis patient with the $Fc_\epsilon R$ molecule-based assay, CAP and Immunodot anti-IgE monoclonal antibody assays, a histamine release assay and a skin test.

Fig. 2 compares the reactivities of the sera of patients with allergy symptoms using a $Fc_\epsilon R$ molecule-based assay and an anti-IgE monoclonal antibody-based assay.

Fig. 3 depicts the correlation between allergen specificities detected by heat-stable $Fc_\epsilon R$ α chain-reactive immunoglobulins and those detected by a pan anti-IgG monoclonal antibody.

DETAILED DESCRIPTION OF THE INVENTION

The present invention includes a method to detect a biologically active immunoglobulin that selectively binds to a specific allergen in a mammal. The method includes the steps of (a) contacting a putative biologically active, allergen-specific immunoglobulin-containing composition from a mammal with an isolated Fc_ϵ receptor ($Fc_\epsilon R$) molecule and with the specific allergen under conditions suitable for formation of

a $Fc_\epsilon R$:immunoglobulin:allergen complex; and (b) determining the presence of the immunoglobulin by detecting the complex. The presence of a complex indicates the presence of such an immunoglobulin in the composition. As such, included in the present invention is the observation that a $Fc_\epsilon R$ molecule of the present invention can indeed bind to an immunoglobulin bound to its specific allergen. The present invention also includes the surprising discovery that such a method permits the detection of biologically active, allergen-specific immunoglobulins that are not detectable, or only weakly detectable, by a process using anti-IgE antibodies. That is, the present invention detects allergen-specific immunoglobulins that *in vitro* assays using (e.g., employing) antibodies raised against the constant region of IgE are not able to detect (e.g., identify, find, observe), or are only able to detect weakly. While not being bound by theory, it is believed that such anti-IgE antibodies are not able to recognize or bind to such immunoglobulins, at least not to an extent that permits their detection to any significantly extent (i.e., there may be no detection or only weak detection of such immunoglobulins). In studies conducted to date, compositions isolated from (i.e., samples collected from) at least about 5% of a population of mammals include biologically active, allergen-specific immunoglobulins not detectable by a process using anti-IgE antibodies. Preferably, compositions isolated from about 10% of a population of mammals include biologically active, allergen-specific immunoglobulins not detectable by a process using anti-IgE antibodies.

As used herein, a biologically active, allergen-specific immunoglobulin is an immunoglobulin, or antibody, that selectively binds to a specific allergen and thereby is able to activate a $Fc_\epsilon RI$ present on a cell surface. As such, the term biologically active refers to the immunoglobulin's ability, upon binding to a specific allergen, to trigger basophil and/or mast cell degranulation, to activate a cell leading to mediator release therefrom, to elicit immediate skin reactivity, and/or to cause other manifestations of allergy. A specific allergen is an allergen that selectively binds to an immunoglobulin that is capable of activating a $Fc_\epsilon RI$. Such an immunoglobulin is referred to herein as an allergen-specific immunoglobulin. As used herein, the term "selectively binds to" refers to the ability of a first molecule to bind to a second molecule in a specific, or

selective, manner. That is, the first molecule preferentially binds to the second molecule as opposed to binding to an unrelated molecule.

An immunoglobulin of the present invention can be IgE or can be IgE-like. An IgE-like immunoglobulin is an immunoglobulin that is biologically similar to IgE in that it can, in the presence of the relevant specific allergen, bind to and activate $Fc_{\epsilon}RI$. IgE-like immunoglobulins are those that are detected by methods of the present invention but not by known processes using anti-IgE antibodies. While not being bound by theory, it is believed that such immunoglobulins may be members of a subclass of IgE that are allergenically relevant but that are not recognized by anti-IgE antibodies or may be immunoglobulins of another isotype that are able to interact with a $Fc_{\epsilon}RI$ in such a manner as to elicit an allergenic response.

One embodiment of the present invention is the surprising discovery of a heat stable biologically active, allergen-specific immunoglobulin; such an immunoglobulin reacts (i.e., binds to, positively reacts with) a $Fc_{\epsilon}R$ molecule of the present invention but not with an anti-IgE monoclonal antibody. As used herein, a heat stable immunoglobulin is an immunoglobulin will bind to a $Fc_{\epsilon}R$ molecule after having been exposed to conditions that would inactivate heat labile IgE molecules; note that it is known to those skilled in the art the IgE molecules are typically heat labile. An example of such conditions is exposure of an immunoglobulin to 56°C for 1 hour. Without being bound by theory, it is believed that such heat stable biologically active, allergen-specific immunoglobulins represent a novel subclass of immunoglobulins, such as a heat-stable IgE immunoglobulin or a $Fc_{\epsilon}R$-reactive IgG immunoglobulin, perhaps an anaphylactic immunoglobulin.

A method of the present invention is advantageous for a number of reasons, including but not limited to the following: such a method detects immunoglobulins that are allergen-specific; such a method detects allergenically relevant immunoglobulins; such a method does not detect non-biologically active IgE; such a method does not detect irrelevant antibodies of other isotypes; and such a method detects biologically active, allergen-specific immunoglobulins in patients having a history of allergic symptoms, even in patients whose serum does not include IgE detectable by a process using anti-IgE antibodies. Furthermore, a method of the present invention does not

require a composition obtained from a mammal for testing to be diluted prior to use. Neither are washing steps required. As such, a composition can either be diluted or not, and washing can either be conducted or not. As such, the present invention is further distinguished from JP Publication 8-101194, *ibid.*, which discusses the importance of sample dilution and washing steps to perform the assay disclosed therein.

One embodiment of the present invention is a method to detect a biologically active, allergen-specific immunoglobulin using an isolated $Fc_\epsilon R$ molecule. It is to be noted that the term "a" entity or "an" entity refers to one or more of that entity; for example, a protein refers to one or more proteins or at least one protein. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. Furthermore, a compound "selected from the group consisting of" refers to one or more of the compounds in the list that follows, including mixtures (i.e., combinations) of two or more of the compounds.

According to the present invention, an isolated, or biologically pure, $Fc_\epsilon R$ molecule, is a molecule that has been removed from its natural milieu; e.g., an isolated $Fc_\epsilon R$ molecule of the present invention has been separated from the cell with which it might naturally occur (e.g., a basophil or mast cell). As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the molecule has been purified. An isolated $Fc_\epsilon R$ molecule of the present invention can be obtained from its natural source (e.g., from a basophil or mast cell), can be produced using recombinant DNA technology or can be produced by chemical synthesis.

A $Fc_\epsilon R$ molecule (also referred to herein as $Fc_\epsilon R$ or $Fc_\epsilon R$ protein) of the present invention can be a full-length protein, a portion of a full-length protein or any homolog of such a protein. As used herein, a protein can be a polypeptide or a peptide. A $Fc_\epsilon R$ molecule of the present invention can include a complete $Fc_\epsilon RI$ (i.e., alpha, beta and gamma $Fc_\epsilon R$ chains), an alpha chain associated with either a beta or gamma chain, an alpha $Fc_\epsilon R$ chain (also referred to herein as $Fc_\epsilon R$ α chain) or a portion of a $Fc_\epsilon R$ α chain. As such, a $Fc_\epsilon R$ molecule includes at least a portion of a $Fc_\epsilon R$ α chain that binds to IgE, i.e., that is capable of forming an immunocomplex with an IgE constant region. A preferred $Fc_\epsilon R$ molecule of the present invention includes a soluble $Fc_\epsilon R$ α chain

(i.e., a $Fc_\epsilon R$ α chain without a functional transmembrane or cytoplasmic domain) or a portion thereof that is capable of binding to IgE. A $Fc_\epsilon R$ molecule of the present invention preferably binds to IgE with an affinity of about $K_A \approx 10^8$, more preferably with an affinity of about $K_A \approx 10^9$ and even more preferably with an affinity of about $K_A \approx 10^{10}$.

An isolated $Fc_\epsilon R$ molecule of the present invention, including a homolog, can be identified in a straight-forward manner by the $Fc_\epsilon R$ molecule's ability to form an immunocomplex with an IgE. Examples of $Fc_\epsilon R$ homologs include $Fc_\epsilon R$ proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the homolog includes at least one epitope capable of forming an immunocomplex with an IgE.

$Fc_\epsilon R$ homologs can be the result of natural allelic variation or natural mutation. $Fc_\epsilon R$ homologs of the present invention can also be produced using techniques known in the art including, but not limited to, direct modifications to the protein or modifications to the gene encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

A preferred $Fc_\epsilon R$ molecule of the present invention is a mammalian $Fc_\epsilon R$ molecule. Examples of suitable mammalian $Fc_\epsilon R$ molecules include, but are not limited to, human, feline, canine, equine, bovine, ovine, porcine, rodent, other companion animal (i.e., pet), economic food animal or zoo animal $Fc_\epsilon R$ molecules, with human or companion animal $Fc_\epsilon R$ molecules being more preferred. Particularly preferred are human, feline, canine, or equine $Fc_\epsilon R$ molecules, with human and canine $Fc_\epsilon R$ molecules being even more preferred. Examples of human, feline, canine, and rat $Fc_\epsilon R$ α chains are disclosed, for example, in US 4,962,035, *ibid.*; US 5,639,660, *ibid.*; Kochan et al, *ibid.*; Shimizu et al., *ibid.*; Pang et al., *ibid.*; PCT Publication No. WO 98/23964, by Frank et al., published June 4, 1998; PCT Publication No. WO 98/27208, by Frank et al., published June 25, 1998; and PCT Publication No. WO 98/45707, by Frank et al., published October 15, 1998. Knowing the nucleic acid and amino acid sequences of such genes and proteins, respectively, enables one skilled in the art to identify other $Fc_\epsilon R$ α chains, including soluble $Fc_\epsilon R$ α chains, and IgE-binding portions thereof, including

those from other mammals. Examples of such techniques are disclosed, for example, by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, 1989. In one embodiment, a particularly preferred $Fc_\epsilon R$ molecule is a $Fc_\epsilon R$ molecule that is highly labeled and stable. Methods to produce such a molecule are disclosed herein.

An isolated $Fc_\epsilon R$ molecule of the present invention can be produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a recombinant cell that is capable of expressing the protein, the recombinant cell being produced by transforming a host cell with one or more nucleic acid molecules encoding a $Fc_\epsilon R$ molecule of the present invention (i.e., a nucleic acid molecule of the present invention). Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained. Suitable and preferred nucleic acid molecules with which to transform a cell are those that encode suitable and preferred $Fc_\epsilon R$ molecules as disclosed herein.

Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule encoding a $Fc_\epsilon R$ molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule. Host cells of the present invention either can be endogenously (i.e., naturally) capable of producing a $Fc_\epsilon R$ molecule protein of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host cells of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal (including yeast), insect, other animal and plant cells.

Preferably, a recombinant cell is transfected with a recombinant molecule of the present invention. A recombinant molecule includes at least one of any nucleic acid molecule heretofore described operatively linked to at least one of any transcription control sequence capable of effectively regulating expression of the nucleic acid molecule(s) in the cell to be transformed.

A $Fc_\epsilon R$ molecule of the present invention can include chimeric molecules including at least a portion of a $Fc_\epsilon R$ molecule that binds to an IgE and a second molecule that enables the chimeric molecule to be bound to a substrate in such a manner that the $Fc_\epsilon R$ portion binds to IgE in essentially the same manner as a $Fc_\epsilon R$ molecule that is not bound to a substrate. An example of a suitable second molecule includes at least a portion of an immunoglobulin molecule or other ligand that either binds directly to a substrate or to its complementary ligand immobilized on a substrate.

A $Fc_\epsilon R$ molecule of the present invention can be bound to the surface of a cell expressing the $Fc_\epsilon R$ molecule. A preferred $Fc_\epsilon R$-bearing cell is a recombinant cell expressing a nucleic acid molecule encoding a $Fc_\epsilon R$ α chain of the present invention.

The present invention also includes $Fc_\epsilon R$ mimetopes and use thereof to detect biologically active, allergen-specific immunoglobulins. In accordance with the present invention, a "mimetope" refers to any compound that is able to mimic the ability of a $Fc_\epsilon R$ molecule to bind to biologically active, allergen-specific immunoglobulins. A mimetope can be a peptide that has been modified to decrease its susceptibility to degradation but that still retains-binding activity. Other examples of mimetopes include, but are not limited to, carbohydrate-based compounds, lipid-based compounds, nucleic acid-based compounds, natural organic compounds, synthetically derived organic compounds, anti-idiotypic antibodies and/or catalytic antibodies, or fragments thereof. A mimetope can be obtained by, for example, screening libraries of synthetic compounds for compounds capable of binding to biologically active, allergen-specific immunoglobulins. A mimetope can also be obtained by, for example, rational drug design. In a rational drug design procedure, the three-dimensional structure of a compound of the present invention can be analyzed by, for example, nuclear magnetic resonance (NMR) or x-ray crystallography. The three-dimensional structure can then be used to predict structures of potential mimetopes by, for example, computer modeling.

The predicted mimetope structures can then be produced by, for example, chemical synthesis, recombinant DNA technology, or by isolating a mimetope from a natural source. Specific examples of $Fc_\epsilon R$ mimetopes include anti-idiotypic antibodies, oligonucleotides produced using Selex technology, peptides identified by random screening of peptide libraries and proteins identified by phage display technology.

A $Fc_\epsilon R$ molecule of the present invention can be contained in a formulation, herein referred to as a $Fc_\epsilon R$ formulation. For example, a $Fc_\epsilon R$ molecule can be combined with a buffer in which the $Fc_\epsilon R$ molecule is solubilized and/or with a carrier. Suitable buffers and carriers are known to those skilled in the art. Examples of suitable buffers include any buffer in which a $Fc_\epsilon R$ molecule can function to selectively bind to a biologically active, allergen-specific immunoglobulin, such as, but not limited to, phosphate buffered saline, water, saline, phosphate buffer, bicarbonate buffer, HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffered saline), TES buffer (Tris-EDTA buffered saline), Tris buffer and TAE buffer (Tris-acetate-EDTA). Examples of carriers include, but are not limited to, polymeric matrices, toxoids, and serum albumins, such as bovine serum albumin. Carriers can be mixed with a $Fc_\epsilon R$ molecule or conjugated (i.e., attached) to a $Fc_\epsilon R$ molecule in such a manner as to not substantially interfere with the ability of the $Fc_\epsilon R$ molecule to selectively bind to a biologically active, allergen-specific immunoglobulin.

As disclosed herein, a specific allergen of the present invention is an allergen that, when it selectively binds to a biologically active, allergen-specific immunoglobulin *in vivo*, triggers an allergic response. Such an allergen can also bind to such an immunoglobulin *in vitro*. A suitable specific allergen is any substance that can induce the production of IgE or IgE-like immunoglobulins. Examples of specific allergens include, but are not limited to, bacterial allergens, fungal allergens, endoparasite allergens, ectoparasite allergens, food allergens, pollen allergens, other animal allergens and other plant allergens. Such specific allergens include, but are not limited to, bacterial, yeast, fungal, heartworm, other helminth, flea, fly, mosquito, mite, midge, biting gnat, lice, bee, wasp, ant, cockroach, true bug, tick, human dander, cat, dog, cattle, poultry, swine, sheep, lamb, dust, tree, weed, shrub, grass, wheat, corn, soybean, peanut, walnut, rice, egg, milk, and cheese allergens, as well as other pollen allergens. Preferred

specific allergens include, but are not limited to, cat, dog, grass, fescue, dock, plantain, firebush, pigweed, ragweed, thistle, rye, olive, hazel, sage, elm, juniper, pine, aspen, cocklebur, sorrel, elder, walnut, cottonwood, ash, birch, cedar, oak, mulberry, olea, parietaria, mugwort, ribwort, milk, egg, peanut, celery, tomato, hazelnut, shrimp, wheat, soja, dust, ash, smut, heartworm, cockroach, flea, *Dermatophagoides*, *Alternaria*, *Aspergillus*, *Candida*, *Cladosporium*, *Fusarium*, *Helminthosporium*, *Mucor*, *Penicillium*, *Pullularia*, *Rhizopus* and *Tricophyton* allergens, such as, but not limited to, Johnson grass, Kentucky blue grass, meadow fescue, orchard grass, perennial rye grass, redtop grass, Timothy grass, June grass, Bermuda grass, brome grass, curly dock, English plantain, Mexican firebush, lamb's quarters, rough pigweed, short ragweed, wormwood sage, American elm, common cocklebur, box elder, black walnut, Eastern cottonwood, green ash, river birch, red cedar, Japanese cedar, red oak, red mulberry, cockroach, *Dirofilaria immitis*, *Ctenocephalides*, *Dermatophagoides pteronyssinus*, *Dermataphagoides farinae*, *Alternaria alternata*, *Aspergillus fumigatus*, *Candida albicans*, *Cladosporium herbarum*, *Fusarium vasinfectum*, *Helminthosporium sativum*, *Mucor recemosus*, *Penicillium notatum*, *Pullularia pullulans*, *Rhizopus nigricans* and *Tricophyton* spp. A preferred flea allergen is a flea saliva allergen, such as those flea saliva products and proteins disclosed in U.S. Patent No. 5,646,115, by Frank et al, issued July 8, 1997.

A specific allergen can be produced from its natural source or can be produced synthetically; a protein allergen can also be produced recombinantly. A specific allergen can be a whole allergen or a portion thereof. The smallest portion is an epitope that is capable of eliciting an immune response against the whole allergen from which the portion is derived.

One embodiment of the present invention is a method to detect a biologically active immunoglobulin that selectively binds to a specific allergen in a mammal. The method includes the steps of: (a) contacting a putative biologically active, allergen-specific immunoglobulin-containing composition from the mammal with an isolated $Fc_\epsilon R$ molecule and with the specific allergen under conditions suitable for formation of a $Fc_\epsilon R$:immunoglobulin:allergen complex; and (b) determining the presence of the

immunoglobulin by detecting the complex, the presence of the complex indicating the presence of the immunoglobulin.

Suitable mammals to test include, but are not limited to, humans, dogs (i.e., canids), cats (i.e., felids), horses (i.e., equids), cattle, sheep, swine, and rodents, as well as other companion animals, food animals, or zoo animals that are mammals. Preferred mammals to test include humans and companion animals, with humans, cats, dogs, and horses being particularly preferred, and humans and dogs being even more preferred. As such, a method of the present invention detects mammalian immunoglobulins, preferably human or companion animal immunoglobulins, more preferably human, feline, canine, and equine immunoglobulins, and even more preferably human and canine immunoglobulins. In one embodiment, such a mammalian immunoglobulin is heat stable, with human or companion animal heat stable immunoglobulins being preferred, with human, feline, canine or equine heat stable immunoglobulins being more preferred, and with human heat stable immunoglobulins being even more preferred.

One embodiment of the present invention is an isolated biologically active, allergen-specific immunoglobulin that is heat stable and that selectively binds to a mammalian $Fc_\epsilon R$ molecule but that preferably does not bind to an anti-IgE monoclonal antibody. As used herein, an isolated, or biologically pure, heat stable immunoglobulin of the present invention is an immunoglobulin that has been removed from its natural milieu. As such, "isolated" and "biologically pure" do not necessarily reflect the extent to which the molecule has been purified. An isolated heat stable immunoglobulin of the present invention can be obtained from its natural source, can be produced using recombinant DNA technology or can be produced by chemical synthesis. In one embodiment, a heat stable immunoglobulin of the present invention can be obtained using a $Fc_\epsilon R$ molecule of the present invention.

As used herein, the term "contacting" refers to combining or mixing, in this case, a putative biologically active, allergen-specific immunoglobulin-containing composition with a mammalian $Fc_\epsilon R$ molecule and a specific allergen. Formation of a complex between a $Fc_\epsilon R$ molecule, a specific allergen and a biologically active, allergen-specific immunoglobulin, i.e., a $Fc_\epsilon R$:immunoglobulin:allergen complex, refers to the ability of the specific allergen and $Fc_\epsilon R$ molecule to selectively bind to the biologically active,

allergen-specific immunoglobulin in order to form a stable complex that can be measured (i.e., detected). Binding between a $Fc_\epsilon R$ molecule, a specific allergen and a biologically active, allergen-specific immunoglobulin is effected under conditions suitable to form a complex; such conditions (e.g., appropriate concentrations, buffers, temperatures, reaction times) as well as methods to optimize such conditions are known to those skilled in the art, and examples are disclosed herein. Examples of complex formation conditions are also disclosed in, for example, in Sambrook et al., *ibid.* It is to be noted that a $Fc_\epsilon R$:immunoglobulin:allergen complex can be formed in a variety of orders, for example, (a) by contacting the specific allergen with the composition and then contacting with the $Fc_\epsilon R$ molecule, (b) by contacting the composition with the $Fc_\epsilon R$ molecule and then with the specific allergen, or (c) by contacting the three at the same time.

As used herein, the term "detecting complex formation" refers to determining if any complex is formed, i.e., assaying for the presence (i.e., existence) of a complex. If complexes are formed, the amount of complexes formed can, but need not be, determined. Complex formation, or selective binding, between a $Fc_\epsilon R$ molecule, a specific allergen, and a biologically active, allergen-specific immunoglobulin in the composition can be measured (i.e., detected, determined) using a variety of methods standard in the art (see, for example, Sambrook et al. *ibid.*), examples of which are disclosed herein.

A putative biologically active, allergen-specific immunoglobulin-containing composition of the present invention refers to a biological sample from a mammal. A suitable biological sample includes, but is not limited to, a bodily fluid composition or a cellular composition. A bodily fluid refers to any fluid that can be collected (i.e., obtained) from a mammal, examples of which include, but are not limited to, blood, serum, plasma, urine, tears, aqueous humor, central nervous system fluid (CNF), saliva, lymph, nasal secretions, milk and feces. A preferred composition of the present method is serum.

A composition of the present method can also include a biologically active, allergen-specific immunoglobulin-producing cell. Such a cell can have such an immunoglobulin bound to the surface of the cell and/or can secrete such an

immunoglobulin. The immunoglobulin can be bound to the surface of a cell either directly to the membrane of a cells or bound to a molecule (e.g., an allergen) bound to the surface of the cell.

A complex can be detected in a variety of ways including, but not limited to use of one or more of the following assays: an enzyme-linked immunoassay, a radioimmunoassay, a fluorescence immunoassay, a chemiluminescent assay, a lateral flow assay, an agglutination assay, a particulate-based assay (e.g., using particulates such as, but not limited to, magnetic particles or plastic polymers, such as latex or polystyrene beads), an immunoprecipitation assay, a BioCore™ assay (e.g., using colloidal gold), an immunodot assay (e.g., CMG's Immunodot System, Fribourg, Switzerland), and an immunoblot assay (e.g., a western blot). Such assays are well known to those skilled in the art. Assays can be used to give qualitative or quantitative results depending on how they are used. Some assays, such as agglutination, particulate separation, and immunoprecipitation, can be observed visually (e.g., either by eye or by a machines, such as a densitometer or spectrophotometer) without the need for a detectable marker. In other assays, conjugation (i.e., attachment) of a detectable marker to the $Fc_\epsilon R$ molecule or to a reagent that selectively binds to the $Fc_\epsilon R$ molecule or to the immunoglobulin being detected (described in more detail below) aids in detecting complex formation. Examples of detectable markers include, but are not limited to, a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label or a ligand. A ligand refers to a molecule that binds selectively to another molecule. Preferred detectable markers include, but are not limited to, fluorescein, a radioisotope, a phosphatase (e.g., alkaline phosphatase), biotin, avidin, a peroxidase (e.g., horseradish peroxidase), beta-galactosidase, and biotin-related compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure® NeutrAvidin).

In one embodiment, a $Fc_\epsilon R$:immunoglobulin:allergen complex is detected by contacting a putative biologically active, allergen-specific immunoglobulin-containing composition with a specific allergen and with a $Fc_\epsilon R$ molecule that is conjugated to a detectable marker. A suitable detectable marker to conjugate to a $Fc_\epsilon R$ molecule includes, but is not limited to, a radioactive label, a fluorescent label, a chemiluminescent label, a chromophoric label, or a ligand. A detectable marker is

conjugated to a $Fc_\epsilon R$ molecule or a reagent in such a manner as not to block the ability of the $Fc_\epsilon R$ or reagent to bind to the biologically active, allergen-specific immunoglobulin being detected. Preferred detectable markers include, but are not limited to, fluorescein, a radioisotope, a phosphatase (e.g., alkaline phosphatase), biotin, avidin, a peroxidase (e.g., horseradish peroxidase), beta-galactosidase, and biotin-related compounds or avidin-related compounds (e.g., streptavidin or ImmunoPure® NeutrAvidin). Preferably, a carbohydrate group of a $Fc_\epsilon R$ molecule, and more preferably of a $Fc_\epsilon R$ α chain, is conjugated to biotin. Also preferable is a $Fc_\epsilon R$ molecule that is highly labeled with a detectable marker.

In another embodiment, a $Fc_\epsilon R$:immunoglobulin:allergen complex is detected by contacting a putative biologically active, allergen-specific-containing composition with a $Fc_\epsilon R$ molecule and with a specific allergen and then contacting the complex with an indicator molecule. Suitable indicator molecules of the present invention include molecules that can bind to the $Fc_\epsilon R$ molecule, to the specific allergen, or to the immunoglobulin. As such, an indicator molecule can comprise, for example, a $Fc_\epsilon R$ molecule, an antigen, an antibody or a lectin, depending upon which portion of the complex being detected. Preferred identifying labeled compounds that are antibodies include, for example, anti-biologically active, allergen-specific immunoglobulin antibodies, anti-$Fc_\epsilon R$ antibodies, and anti-allergen antibodies. Preferred lectins include those lectins that bind to high mannose-containing groups present on a member of the complex. More preferred lectins bind to high mannose-containing groups present on a $Fc_\epsilon R$ molecule of the present invention produced in insect cells. An indicator molecule itself can be attached to a detectable marker of the present invention. For example, an antibody can be conjugated to biotin, horseradish peroxidase, alkaline phosphatase or fluorescein.

In one embodiment, a $Fc_\epsilon R$:immunoglobulin:allergen complex is detected by contacting the complex with a reagent that selectively binds to a $Fc_\epsilon R$ molecule of the present invention. Examples of such a reagent include, but are not limited to, an antibody that selectively binds to a $Fc_\epsilon R$ molecule (referred to herein as an anti-$Fc_\epsilon R$ antibody) or a compound that selectively binds to a detectable marker conjugated to a $Fc_\epsilon R$ molecule. $Fc_\epsilon R$ molecules conjugated to biotin are preferably detected using

streptavidin, more preferably using ImmunoPure® NeutrAvidin (available from Pierce, Rockford, IL).

In another embodiment, a $Fc_\epsilon R$:immunoglobulin:allergen complex is detected by contacting the complex with a reagent, such as an antibody or other ligand, that selectively binds to the specific allergen to which the biologically active, allergen-specific immunoglobulin is bound. Such a reagent may itself contain a detectable marker or can be detected with yet another indicator molecule that binds to that reagent.

The present invention can further comprise one or more layers and/or types of secondary molecules or other binding molecules capable of detecting the presence of an indicator molecule. For example, an untagged (i.e., not conjugated to a detectable marker) secondary antibody that selectively binds to an indicator molecule can be bound to a tagged (i.e., conjugated to a detectable marker) tertiary antibody that selectively binds to the secondary antibody. Suitable secondary antibodies, tertiary antibodies and other secondary or tertiary molecules can be selected by those of skill in the art. Preferred tertiary molecules can be selected by a skilled artisan based upon the characteristics of the secondary molecule. The same strategy is applied for subsequent layers.

In one embodiment, a $Fc_\epsilon R$:immunoglobulin:allergen complex can be formed and detected in solution.

In another embodiment, a $Fc_\epsilon R$:immunoglobulin:allergen complex can be formed in which one or more members of the complex are immobilized on (e.g., coated onto) a substrate. Immobilization techniques are known to those skilled in the art. Suitable substrate materials include, but are not limited to, plastic, glass, gel, celluloid, fabric, paper, and particulate materials. Examples of substrate materials include, but are not limited to, latex, polystyrene, nylon, nitrocellulose, agarose, cotton, PVDF (poly-vinylidene-fluoride), and magnetic resin. Suitable shapes for substrate material include, but are not limited to, a well (e.g., microtiter dish well), a plate, a dipstick, a strip, a bead, a lateral flow apparatus, a membrane, a filter, a tube, a dish, a celluloid-type matrix, a magnetic particle, and other particulates. A particularly preferred substrate comprises an ELISA plate, a dipstick, an immunodot strip, a radioimmunoassay plate, an agarose bead, a plastic bead, a latex bead, a sponge, a cotton thread, a plastic chip, an

immunoblot membrane and an immunoblot paper. In one embodiment, a substrate, such as a particulate, can include a detectable marker.

A preferred method of the present invention includes a step of: (a) binding a $Fc_\epsilon R$ molecule to a substrate to form a $Fc_\epsilon R$ molecule-coated substrate prior to contacting the $Fc_\epsilon R$ molecule with a putative immunoglobulin-containing composition and a specific allergen; (b) binding a specific allergen to a substrate to form an allergen-coated substrate prior to contacting the allergen with a putative immunoglobulin-containing composition and a $Fc_\epsilon R$ molecule; or (c) binding a putative immunoglobulin-containing composition to a substrate to form a putative immunoglobulin-containing composition-coated substrate prior to contacting the composition with a $Fc_\epsilon R$ molecule or specific allergen. In the latter case, the substrate can be a non-coated substrate, but is preferably a $Fc_\epsilon R$ molecule-coated substrate or an allergen-coated substrate.

In a preferred embodiment, a specific allergen is immobilized on a substrate, such as a microtiter dish well, a dipstick, an immunodot strip, or a lateral flow apparatus. Preferred allergens include those disclosed herein. A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable (i.e., sufficient) to allow for immunoglobulin:allergen complex formation bound to the substrate (i.e., immunoglobulin in the sample binds to allergen immobilized on the substrate). A $Fc_\epsilon R$ molecule is added to the substrate and incubated to allow for formation of a complex between the $Fc_\epsilon R$ molecule and the immunoglobulin:allergen complex. Preferably, the $Fc_\epsilon R$ molecule is conjugated to a detectable marker. A developing agent is added, if required, and the substrate is submitted to a detection device for analysis. In some protocols, washing steps are added after one or both complex formation steps in order to remove excess reagents. If such steps are used, they involve conditions known to those skilled in the art such that excess reagents are removed but the complex is retained on the substrate. Preferred conditions are disclosed herein in the Examples section and generally in Sambrook et al., *ibid.*

In another embodiment, a $Fc_\epsilon R$ molecule is immobilized on a substrate, such as a microtiter dish well, a dipstick, an immunodot strip, or a lateral flow apparatus. A biological sample collected from an animal is applied to the substrate and incubated under conditions suitable to allow for $Fc_\epsilon R$:immunoglobulin formation bound to the

substrate. A specific allergen is added to the substrate and incubated to allow for formation of a complex between the allergen and the $Fc_\epsilon R$:immunoglobulin complex. Preferred allergens are disclosed herein. In one embodiment, the allergen is conjugated to a detectable marker. In another embodiment, an indicator molecule, preferably conjugated to a detectable marker, that can selectively bind to the allergen is added to the substrate. A developing agent is added, if required, and the substrate is submitted to a detection device for analysis. In some protocols, washing steps are added after one or both complex formation steps in order to remove excess reagents.

A preferred method to detect biologically active, allergen-specific immunoglobulins is a lateral flow assay, examples of which are disclosed in U.S. Patent No. 5,424,193, issued June 13, 1995, by Pronovost et al.; U.S. Patent No. 5,415,994, issued May 16, 1995, by Imrich et al; WO 94/29696, published December 22, 1994, by Miller et al.; and WO 94/01775, published January 20, 1994, by Pawlak et al. A lateral flow assay is an example of a single-step assay. In one embodiment, a biological sample is placed in a lateral flow apparatus that includes the following components: (a) a support structure defining a flow path; (b) a labeling reagent comprising a bead conjugated to a specific allergen, the labeling reagent being impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising a $Fc_\epsilon R$ molecule. Preferred specific allergens include those disclosed herein. The capture reagent is located downstream of the labeling reagent within a capture zone fluidly connected to the labeling zone in such a manner that the labeling reagent can flow from the labeling zone into the capture zone. The support structure comprises a material that does not impede the flow of the beads from the labeling zone to the capture zone. Suitable materials for use as a support structure include ionic (i.e., anionic or cationic) material. Examples of such a material include, but are not limited to, nitrocellulose, PVDF, or carboxymethylcellulose. The support structure defines a flow path that is lateral and is divided into zones, namely a labeling zone and a capture zone. The apparatus can further comprise a sample receiving zone located along the flow path, more preferably upstream of the labeling reagent. The flow path in the support structure is created by contacting a portion of the support structure downstream of the capture

zone, preferably at the end of the flow path, to an absorbent capable of absorbing excess liquid from the labeling and capture zones.

In this embodiment, the biological sample is applied to the sample receiving zone which includes a portion of the support structure. The labeling zone receives the sample from the sample receiving zone which is directed downstream by the flow path. The labeling zone comprises the labeling reagent that binds to biologically active, allergen-specific immunoglobulins in the sample. A preferred labeling reagent is an allergen conjugated, either directly or through a linker, to a plastic bead substrate, such as to a latex bead. The substrate also includes a detectable marker, preferably a colorimetric marker. Typically, the labeling reagent is impregnated to the support structure by drying or lyophilization. The sample structure also comprises a capture zone downstream of the labeling zone. The capture zone receives labeling reagent from the labeling zone which is directed downstream by the flow path. The capture zone contains the capture reagent, in this case a $Fc_{\epsilon}R$ molecule, as disclosed above, that immobilizes the immunoglobulin complexed to the allergen in the capture zone. The capture reagent is preferably fixed to the support structure by drying or lyophilizing. The labeling reagent accumulates in the capture zone and the accumulation is assessed visually or by an optical detection device.

In another embodiment, a lateral flow apparatus used to detect biologically active, allergen-specific immunoglobulins includes: (a) a support structure defining a flow path; (b) a labeling reagent comprising a $Fc_{\epsilon}R$ molecule as described above, the labeling reagent impregnated within the support structure in a labeling zone; and (c) a capture reagent comprising a specific allergen, the capture reagent being located downstream of the labeling reagent within a capture zone fluidly connected to the labeling zone in such a manner that the labeling reagent can flow from the labeling zone into the capture zone. The apparatus preferably also includes a sample receiving zone located along the flow path, preferably upstream of the labeling reagent. The apparatus preferably also includes an absorbent located at the end of the flow path.

Another preferred method to detect biologically active, allergen-specific immunoglobulins is an immunodot strip assay, such as is employed in a CMG Immunodot System. In this assay, one or more specific allergens are spotted onto a nitrocellulose strip. Preferred allergens include those disclosed herein. A biological

sample collected from an animal is applied to the strip and incubated under conditions suitable (i.e., sufficient) to allow for immunoglobulin:allergen complex formation bound to the strip. A $Fc_\epsilon R$ molecule is added to the strip and incubated to allow for formation of a complex between the $Fc_\epsilon R$ molecule and the immunoglobulin:allergen complex. Preferably, the $Fc_\epsilon R$ molecule is conjugated to a detectable marker. A developing agent is added, if required, and the substrate is submitted to a detection device for analysis. This assay can be a dual-step or multiple-step assay, as desired.

The present invention also includes kits to detect biologically active, allergen-specific immunoglobulins based on the disclosed detection methods. One embodiment is a kit to detect a biologically active, allergen-specific immunoglobulin that includes a mammalian $Fc_\epsilon R$ molecule, a specific allergen, and a means for detecting a biologically active, allergen-specific immunoglobulin. Suitable and preferred $Fc_\epsilon R$ molecules are disclosed herein. Suitable means of detection include compounds disclosed herein that bind to the $Fc_\epsilon R$ molecule, to the allergen, or to the immunoglobulin. As such, a kit can also comprise a detectable marker, an antibody that selectively binds to the $Fc_\epsilon R$ molecule or to the specific allergen, or other indicator molecules.

A kit can comprise one or more specific allergens. If a kit includes two or more allergens, the allergens can be in formulations such that the allergens remain separate or they can be combined in one or more groups.

A preferred kit of the present invention is one in which the allergen(s) or the $Fc_\epsilon R$ molecule is immobilized on a substrate. Such a kit can contain one or more substrates which can be joined together, for example, in a fan-like format.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

Example 1

This example describes detection of biologically active, allergen-specific immunoglobulins in humans using a $Fc_\epsilon R$ molecule of the present invention. This example also compares results obtained using the $Fc_\epsilon R$ molecule with those obtained using anti-IgE monoclonal antibodies.

Sera collected from 188 allergic patients and 53 control patients (patients who scored negative by intradermal skin testing) were tested against a variety of allergens using $Fc_\epsilon R$ α chain-based and anti-IgE monoclonal antibody-based assays. The $Fc_\epsilon R$ α chain-based assay used $PhFc_\epsilon R\alpha_{172}$-BIOT, a biotinylated human soluble $Fc_\epsilon R$ α chain, produced as described in PCT Publication No. WO 98/23964, by Frank et al., *ibid.* The immunodot strip anti-IgE-monoclonal antibody-based assay used a mixture of anti-human IgE monoclonal antibodies IT6H10, available from Immunotech, Marseille, France, and 4F4, available from CMG; see also Samoilovich et al, *ACI News 4*, 21-25, 1992. The other anti-IgE monoclonal antibody-based assay was Pharmacia's CAP assay (CAP is a trademark of Pharmacia-Upjohn), which was performed according to manufacturer's protocols.

CMG immunodot strips (available from CMG, Fribourg, Switzerland) were produced using standard procedures (e.g., Hong et al, *J. Immunol. Methods 95*, 195-202, 1986; de Weck et al, *Rev. F. Allergol. 33*, 13-21, 1993) with at least one of the following allergens spotted on per strip: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Penicillium notatum*, *Candida albicans*, cockroach, cat, dog, 6 grass mix, rye, olive, birch, oak, hazel, olea, parietaria, Japanese cedar, mugwort, ribwort, milk, egg, peanut, celery, tomato, hazelnut, shrimp, wheat, and soja allergens.

For the $Fc_\epsilon R$ α chain-based assay, a sample of one milliliter (ml) of undiluted serum was incubated for about 2 hours at room temperature with an immunodot strip spotted with one or more allergens, after which the strip was washed three times for about 5 minutes each with TBS (50 mM Tris, 150 mM NaCl pH 8.0) at room temperature. The strip was then incubated for about 1 hour at room temperature with an about 1:8000 dilution of $PhFc_\epsilon R\alpha_{172}$-BIOT, produced as described above, in one ml TBS. The strip was then washed three times for about 5 minutes each with TBS at room temperature. The strip was then developed by methods known to those skilled in the art, e.g., by incubating with a streptavidin-labeled horse-radish peroxidase for about 30 minutes at room temperature, washed with TBS three times for about 5 minutes each at room temperature, and incubating for 15 minutes at room temperature with an enzyme substrate using a method similar to that described in PCT Publication

No. WO 98/23964, by Frank et al., *ibid*, except adapted for strips. The strip was then washed with distilled water and analyzed.

For the immunodot strip anti-IgE monoclonal antibody-based assay, a sample of one ml of undiluted serum was incubated for about 2 hours at room temperature with an immunodot strip spotted with one or more allergens, after which the strip was washed three times for about 5 minutes each with TBS at room temperature. The strip was then incubated for about 1 hour at room temperature with 1:2000 dilution of 0.5 mg/ml of the anti-human IgE monoclonal antibody mixture in one ml TBS. The strip was then washed three times for about 5 minutes each with TBS at room temperature. The strip was then developed for about 15 minutes at room temperature with a mixture of 2,4 chloronapthol (available from Merck) and hydrogen peroxide using a method as described in Houg et al, *ibid*. The strip was then washed with distilled water and analyzed.

Correlations obtained comparing the $Fc_\epsilon R$ α chain-based assay, the two anti-IgE monoclonal antibody-based assays, and skin tests for given allergens are presented in Tables 1-4. "n" refers to the number of patients. "r" value is the correlation analysis value.

Table 1. Comparison of the $Fc_\epsilon R$ α chain-based assay and the immunodot strip anti-IgE monoclonal antibody-based assay for given allergens

n	Allergen	"r" value
9	olea	0.99
9	*Alternaria*	0.98
81	*D. pteronyssinus*	0.97
81	*D. farinae*	0.97
81	cat	0.97
59	6 grasses mix	0.94
59	rye	0.93
81	dog	0.91
59	ribwort	0.77
59	mugwort	0.57
59	birch	0.11

Table 2. Comparison of the $Fc_\epsilon R$ α chain-based assay and the CAP anti-IgE monoclonal antibody-based assay for given allergens.

n	Allergen	"r" value
73	*D. pteronyssinus*	0.87
58	6 grasses mix	0.70

Table 3. Comparison of the immunodot strip anti-IgE monoclonal antibody-based assay and the CAP anti-IgE monoclonal antibody-based assay for given allergens.

n	Allergen	"r" value
73	*D. pteronyssinus*	0.85
57	6 grasses mix	0.75

Table 4. Comparison of serological assays to skin tests for given allergens

Serological assay	n	Allergen	"r" value
$Fc_\epsilon R$ α chain	73	*D. pteronyssinus*	0.41
	57	6 grasses mix	0.19
anti-IgE: Immunodot	73	*D. pteronyssinus*	0.31
	57	6 grasses mix	0.07
anti-IgE: CAP	73	*D. pteronyssinus*	0.27
	57	6 grasses mix	0.17

Analysis of the results presented in Tables 1-3 indicates that while there is strong correlation between $Fc_\epsilon R$ molecule-based assays and anti-IgE antibody assays for several of the allergens, there is extremely poor correlation for other allergens. In many of the patients showing poor correlation, such poor correlation was due to the ability of the $Fc_\epsilon R$ molecule-based assay to detect biologically active, allergen-specific immunoglobulins that the anti-IgE antibody-based assays did not detect. Furthermore, Table 4 suggests that the $Fc_\epsilon R$ molecule-based assay correlates better with skin testing than do either of the anti-IgE antibody-based assay analyzed.

Table 5 summarizes results for the sera of 16 patients for which only the $Fc_\epsilon R$ molecule-based assay ($Fc_\epsilon R$) detected immunoglobulins or for which the $Fc_\epsilon R$ molecule-based assay detected a significantly higher level of reactivity than did the anti-

IgE antibody-based assays (i.e., immunodot (Dot) or CAP); reactivities are expressed as arbitrary optical density (O.D.) units. It is to be noted that in the other allergic patients, anti-IgE antibody-based assays routinely gave higher reactivities than did the $Fc_{\epsilon}R$ molecule-based assay. Those samples in which the $Fc_{\epsilon}R$ molecule-based assay detected significant levels of immunoglobulin but the anti-IgE antibody assays detected little if any immunoglobulin are highlighted by bolding of the $Fc_{\epsilon}R$ molecule-based assay results.

Table 5. Comparison of the $Fc_\epsilon R$ α chain-based assay and anti-IgE antibody-based assays for patients exhibiting a reaction against at least one allergen for which the $Fc_\epsilon R$ α chain-based assay showed increased reactivity

Patient	Allergen/Assay										
	D. pteronyssinus			*D. farinae*		cat		dog		cockroach	
	$Fc_\epsilon R$	Dot	CAP	$Fc_\epsilon R$	Dot	$Fc_\epsilon R$	Dot	$Fc_\epsilon R$	Dot	$Fc_\epsilon R$	Dot
1375	16	21	61.2	13	19	2	0	2	0	4	1
1392	42	55	100	36	45	15	23	0	0	6	0
349	7	25	0	5	0	0	0	0	0	9	0

	6 grasses			rye		birch		mugwort		ribwort	
	$Fc_\epsilon R$	Dot	CAP	$Fc_\epsilon R$	Dot	$Fc_\epsilon R$	Dot	$Fc_\epsilon R$	Dot	$Fc_\epsilon R$	Dot
1520	76	91	100	67	78	45	0	30	4	45	27
832	25	31	16.3	38	22	0	0	2	0	16	5
840	37	48	26.1	28	11	3	0	3	0	12	0
899	47	59	41.8	48	48	0	0	0	0	12	3
922	42	55	36	44	47	5	0	3	0	12	2
408	4	0	3.2	3	0	4	0	3	0	4	0
396	34	49	20.9	26	29	7	0	7	0	16	0
336	35	50	7.5	23	31	5	0	5	0	5	0
273	45	5	6.3	44	5	27	0	13	0	48	0
242	67	98	101	54	76	11	3	12	4	25	19

	egg			peanut			wheat			milk	
	$Fc_\epsilon R$	Dot	CAP	$Fc_\epsilon R$	Dot	CAP	$Fc_\epsilon R$	Dot	CAP	$Fc_\epsilon R$	Dot
157	5	18	3	8	0		0	1		0	0
10393	0	0		13	0	4.2	8	6		0	0
1149	2	0		3	0		6	0	25	0	0

The results indicate that at least several of the patients whose sera demonstrated a particularly marked reactivity in the $Fc_\epsilon R$ molecule-based assay to a specific allergen demonstrated such reactivity against more than one allergen; see, for example, patients #1375, #349, #1520, #832, #840, #922, #408, #396, #336, #273, #242, and #1149. One patient, #273, exhibited biologically active, allergen-specific immunoglobulins against each of the specific allergens tested (i.e., 6 grasses mix, rye, birch, mugwort, and ribwort) that were detectable with the $Fc_\epsilon R$ molecule-based assay but that were either

not detected or only very weakly detected using the anti-IgE antibody-based assays. Further testing using allergens from *D. pteronyssinus* and plantain indicated that the patient's biologically active, *D. pteronyssinus* allergen-specific immunoglobulins and biologically active, plantain allergen-specific immunoglobulins were similarly detected; see Fig. 1.

Overall, of 140 patients clearly allergic to indoor or outdoor allergens, 13 (i.e., 9.3%) showed positive results with the $Fc_\epsilon R$ molecule-based assay and either negative or weak results with the anti-IgE antibody-based assays. Of 53 patients investigated for suspicion of allergy but with a negative skin test, 3 (i.e., 5.6%) showed tested positive with the $Fc_\epsilon R$ molecule-based assay.

In summary, these results indicate that there is a population of allergic individuals, apparently about 5-10%, that produce biologically active, allergen-specific immunoglobulins that are detected by a $Fc_\epsilon R$ molecule-based assay but not by anti-IgE antibody-based methods.

Example 2

This example describes detection of biologically active, allergen-specific immunoglobulins in dogs using a $Fc_\epsilon R$ molecule of the present invention. This example also compares results obtained using the $Fc_\epsilon R$ molecule with those obtained using anti-IgE monoclonal antibodies.

Sera collected from clinically atopic dogs, experimentally-sensitized dogs, and control dogs (dogs who scored negative by intradermal skin testing) were tested against a variety of allergens using a $Fc_\epsilon R$ α chain-based assay and an immunodot strip anti-IgE-monoclonal antibody-based assay as described in Example 1.

In a first study, immunodot strips were prepared as described in Example 1 with at least one of the following allergens being spotted on per strip: Bermuda grass, June grass, Timothy grass, orchard grass, mugwort, rye, fescue, oak, Japanese cedar, birch, hazel, plantain, house dust mites, storage mites, cat dander, dat flea, *Alternaria*, ovalbumin, peanut, and milk proteins. When the sera of 15 experimentally-sensitized high IgE responder dogs were analyzed for immunoglobulins for specific allergens using the $Fc_\epsilon R$ α chain-based assay and the immunodot anti-IgE monoclonal antibody-based assay, the calculated correlation value was $r=0.96$. However, when the sera of 111

clinically atopic dogs were analyzed for immunoglobulins for specific allergens using the $Fc_\epsilon R$ α chain-based assay and the immunodot anti-IgE monoclonal antibody-based assay, the calculated correlation value was only r=0.37. The poor correlation was often due to the ability of the $Fc_\epsilon R$ α chain-based assay to detect biologically active, allergen-specific allergens which the anti-IgE antibody-based assay did not detect.

In a second study, an enzyme-linked immunoabsorbent assay (ELISA) was conducted in a manner similar to that described in PCT Publication No. WO 98/23964, by Frank et al., *ibid.*, with at least one of the following allergens being added per well: Bermuda grass, June grass, Timothy grass, orchard grass, mugwort, rye, fescue, oak, cedar, birch, elder, cottonwood, elm, juniper, pine, walnut, aspen, ashmix, smut, cockle, dock, sorrel, pigweed, thistle, ragweed, red cedar, house dust mite, EP and LQ. Examples of dogs which scored positive with the $Fc_\epsilon R$ α chain-based assay but negative or weakly with the anti-IgE antibody-based assay are shown in Table 6.

Table 6. Comparison of the $Fc_{\epsilon}R$ α chain-based assay (FC) and the immunodot strip anti-IgE monoclonal antibody-based assay (MAbs) for given allergens.

	Bermuda	June	orchard	P. rye	Timothy	fescue	cockle	dock	sorrel	EP	LQ	pigweed	thistle	ragweed	r. cedar	HDM	mugwort	oak	
1371	0.138	0.254	0.120	0.083	0.162	0.122	0.191	0.194	0.104	0.122	0.087	-0.042					0.005		FC
1371	-0.017	-0.025	-0.017	0.033	-0.015	-0.022	0.022	0.056	0.045	0.028	0.048	0.088					-0.027		Mabs
5796	0.178	0.085	0.107	0.078	0.090	0.091	0.289	0.152	0.080	0.277	0.188	0.087							FC
5796	-0.019	-0.012	-0.007	-0.028	-0.019	-0.017	-0.007	-0.021	-0.024	-0.022	-0.003	-0.030							Mabs
9448	0.212	0.203	0.105	0.168	0.119	0.175	0.276?	0.960?	0.575	0.378?	0.434	0.206							FC
9448	0.035	-0.018	-0.024	-0.006	-0.005	-0.016	-0.015?	[illegible]	0.004	-0.014?	-0.009	0.002							Mabs
5435	0.631?	0.674	[illegible]	[illegible]	0.574?	0.600?	[illegible]	[illegible]	0.577?	0.632?	0.290?	0.619			1.463			0.628?	FC
5435	0.000?	-0.028	0.012?	0.010?	0.002?	0.001?	[illegible]	0.011?	-0.008?	-0.018	0.015?	-0.019			-0.009?			0.025?	Mabs
1992	0.282?	0.311?	[illegible]	0.388?	[illegible]	[illegible]	0.241?	0.245?	0.210?	0.309?	0.133?	0.095							FC
1992	0.033?	0.102?	0.190?	0.178?	0.096?	0.172?	0.015?	0.076?	0.025?	0.005?	0.001?	0.027							Mabs
5781	0.537?	0.214	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2.257?	[illegible]	2.048?							FC
5781	0.021?	0.005	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.030?	0.008?	0.021?							Mabs
r. cedar													2.592	4.140	3.559?	1.558			FC
r. cedar													0.623	0.597	0.225?	0.729			Mabs
NDP	0.210	0.132	0.156	0.099	0.161	0.270	0.112	0.362	0.146	0.317	0.169	0.113							FC
NDP	0.361	0.678	0.541	0.268	0.365	0.488	0.295	0.244	0.266	0.384	0.422	0.198							Mabs
CDP	0.727	1.478	1.467	1.492	1.457	1.956	1.835	0.568	0.499	0.811	0.873?	0.427							FC
CDP	0.421	1.226	1.033	1.136	0.872	1.274	0.764	0.436	0.449	0.323	0.233?	0.236							Mabs
#5	2.178	1.977	2.545	2.382	2.042	2.487	3.057	2.016	2.044	2.213	1.783?	1.168?							FC
#5	1.296	1.989	0.641	1.104	1.503	1.182	0.536	0.728	0.834	1.263	0.245?	0.143?							Mabs
674-85	0.932	0.822	0.711	0.745	0.821	0.656	1.244	0.655	0.869	1.494	1.038	0.689							FC
674-85	0.531	0.546	0.543	0.538	0.506	0.651	0.298	0.552	0.629	0.361	0.329	0.355							Mabs

	W. birch	B. elder	Cottwd	Ch elm	C. elm	juniper	W. oak	W. pine	walnut	aspen	ashmix	smut	
1507	4.140	4.140	4.140	4.140	4.140	[illegible]	4.140	[illegible]	4.140	4.140	4.140	0.854	Fc
1507	0.008	0.012	0.006	0.016?	0.020	0.011	0.012	0.033?	0.014?	0.020	0.007	-0.004	Mabs

1371 = 3+ mugwort, 2+ birch, 2+ June, 2+ orchard by IDST
5796 = 2+ Timothy by IDST
9448 - 3+ rye by IDST
5781 = IDST -ve to allergens in Top Screen Panel
5435 = 1+ June, 1+ fescue, 1+ oak, 4+ cedar by IDST
NDP = Negative Dog Pool

Red cedar - positive dog pool
CDP - Control Dog Pool
#5 - Immunized dog
674-85 = Pooled positive dog sera
HDM = house dust mite
Shading indicates samples with high reactivity according to FC, but not MAbs, assay

These results indicate that dogs also produce biologically active, allergen-specific immunoglobulins that are detectable using $Fc_\epsilon R$-based assays but that are not detectable using anti-IgE antibody-based assays.

Example 3

This Example describes additional studies to detect biologically active, allergen-specific immunoglobulins in humans using a $Fc_\epsilon R$ molecule of the present invention. This Example also identifies biologically active, allergen-specific immunoglobulins in serum samples from a subset of patients that exhibit reactivity with $Fc_\epsilon R$ molecules but not with anti-IgE monoclonal antibodies.

Sera collected from 200 IgE positive patients and 50 IgE negative, but allergen symptomatic, patients were tested against a variety of indoor and outdoor allergens using a $Fc_\epsilon R$ α chain-based assay and an immunodot strip anti-IgE monoclonal antibody-based assay as described in Example 1. Tables 7 and 8 exhibit the correlation between the two assays for indoor (Table 7) and outdoor (Table 8) allergens. Strong reactivities (OD > 10) are denoted by "++"; weak reactivities (OD 2-10) are denoted by "+"; and negative reactivities (OD < 2) are denoted by "-".

Table 7. Correlation between results of a $Fc_\epsilon R$ α chain-based assay ($Fc_\epsilon R$) and an immunodot strip anti-IgE monoclonal antibody-based assay (MAb) to detect allergen-specific immunoglobulins raised against indoor allergens

		$Fc_\epsilon R$		
		++	+	-
MAb	++	56	20	2
	+	1	18	15
	-	1	6	218

Table 8. Correlation between results from a $Fc_\epsilon R$ α chain-based assay ($Fc_\epsilon R$) and an immunodot strip anti-IgE monoclonal antibody-based assay (MAb) to detect allergen-specific immunoglobulins raised against outdoor allergens

		$Fc_\epsilon R$		
		++	+	-
MAb	++	167	21	4
	+	3	22	31
	-	7	22	265

Of 337 reactions conducted using indoor allergens, 7 (i.e., 2.1%), exhibited positive reactivity using the $Fc_\epsilon R$ α chain-based assay and negative reactivity using the anti-IgE monoclonal antibody-based assay. Of 542 reactions conducted using outdoor allergens, 29 (i.e., 5.3%), exhibited positive reactivity using the $Fc_\epsilon R$ α chain-based assay and negative reactivity using the anti-IgE monoclonal antibody-based assay. Of the 200 patients, 17 showed such discrepancies in reactivity (i.e., $Fc_\epsilon R$ positive but MAb negative) for ribwort allergen and 3 showed such discrepancies in reactivity for cockroach allergen. Such discrepancies were reproducible upon repeated serum sample collection from patients and testing, even after several months.

Example 4

This Example demonstrates that a certain subset of patients with allergy symptoms displays positive reactivity in a $Fc_\epsilon R$ α chain-based assay but displays no reactivity in anti-IgE monoclonal antibody-based assays.

Serum samples from fifty patients that had symptoms of allergy but that scored negative when tested using an anti-IgE monoclonal antibody-based assay (i.e., RAST) were tested using a $Fc_\epsilon R$ α chain-based assay as described in Example 1. Allergens used included outdoor allergens, indoor allergens, two mixtures of food allergens, and mold (mould) allergens. Samples from 8 of the 50 patients (i.e., 16%) exhibited $Fc_\epsilon R$ α chain positive reactivity for at least for some of the allergens, as shown in Fig. 2; each patient is indicated by a patient number. The clinical features of these patients is outlined in Table 9.

Table 9. Clinical features of patients reactive with a $Fc_\epsilon R$ α chain but not with anti-IgE monoclonal antibodies

No.	Clinical diagnosis	Age	FcER pos.	Skin tests	RAST	TS
S 731	Perennial rhinitis asthma	18	O,FA,FB (12)	all neg.	all neg.	all neg.
S 1011	Perennial rhinitis recidiv. urticaria	20	O,FA,FB (12)	all neg.	all neg.	all neg.
S 798	Seasonal rhinitis atopic dermatitis, familial atopy	9	O,I,FA,FB (5)	Grass pos.	all neg.	all neg.
S 1364	Chronic sinusitis anesthetics intol.	53	O,FA,FB (27)	Grass pos.	all neg.	all neg.
S 637	Perennial rhinitis	28	O,FC (7)	Some foods pos.	all neg.	all neg.
S 647	Perennial rhinitis	70	O,FC (7)	Some foods pos.	all neg.	all neg.
S 1362	Eczematid	80	O,FB,FC (11)	not done	all neg.	all neg.
S 744	Rhinitis and polyposis aspirin intolerance ?	41	O,I,FA,FB (51)	all neg.	all neg.	all neg.

TS = TOPSCREEN with anti-IgE antibodies
O = outdoor allergens; I = indoor allergens; FA/FB/FC = food allergens; Number in parenthesis = maximal O.D.
pos. = positive; neg. = negative

These patients are particularly interesting because they have biologically active, allergen-specific immunoglobulins in their sera that react with $Fc_\epsilon R$ molecules but not with anti-IgE monoclonal antibodies.

Example 5

This Example demonstrates additional properties of biologically active, allergen-specific immunoglobulins of the present invention that react with $Fc_\epsilon R$ molecules but not with anti-IgE monoclonal antibodies.

Biologically active, allergen-specific immunoglobulins from the eight patients described in Example 4 were tested for heat stability, i.e., whether the $Fc_\epsilon R$-reactive immunoglobulins in the sera of such patients would continue to be active (i.e., bind $Fc_\epsilon R$ α chain) after exposure to heating at 56°C for 1 hour, a condition that typically inactivates IgE binding activity. It is known, for example, by one skilled in the art that the IgE receptor binding domain of IgE immunoglobulins is particularly heat labile.

The serum of each of the eight patients was shown to contain heat stable biologically active, allergen-specific immunoglobulins; i.e., the sera continued to show positive reactivity in a $Fc_{\epsilon}R$ α chain-based assay after exposure of the sera to 56°C for 1 hour at levels similar to that shown by unheated sera.

An experiment was then conducted to determine how the allergen specificities detected by the heat-stable $Fc_{\epsilon}R$ α chain-reactive immunoglobulins correlated with the allergen specificities detected by a pan anti-IgG monoclonal antibody (i.e., a monoclonal antibody that recognizes all IgG subclasses; denoted in Fig. 3 as anti-IgG moAb). The results are depicted in Fig. 3, which indicates there is a positive correlation, particularly for pollen, mite and food allergens, but not for mold (denoted in the figure as mould) allergens.

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims.

What is claimed is:

1. A method to detect a biologically active immunoglobulin that selectively binds to a specific allergen in a mammal, said method comprising:

(a) contacting a putative biologically active, allergen-specific immunoglobulin-containing composition from said mammal with an isolated mammalian Fc_ϵ receptor ($Fc_\epsilon R$) molecule and with said specific allergen under conditions suitable for formation of a $Fc_\epsilon R$:immunoglobulin:allergen complex; and

(b) determining the presence of said immunoglobulin by detecting said complex, the presence of said complex indicating the presence of said immunoglobulin.

2. A method to detect a biologically active, allergen-specific immunoglobulin in a mammal, wherein a process using anti-IgE antibodies does not detect said immunoglobulin, said method comprising:

(a) contacting a putative biologically active, allergen-specific immunoglobulin-containing composition from said mammal with an isolated mammalian $Fc_\epsilon R$ molecule and with said specific allergen under conditions suitable for formation of a $Fc_\epsilon R$:immunoglobulin:allergen complex; and

(b) determining the presence of said immunoglobulin by detecting said complex, the presence of said complex indicating the presence of said immunoglobulin.

3. A kit for detecting a biologically active immunoglobulin in a composition, said immunoglobulin selectively binding to a specific allergen, said kit comprising a mammalian $Fc_\epsilon R$ molecule, said allergen, and a means for detecting said immunoglobulin.

4. An isolated biologically active, allergen-specific immunoglobulin, wherein said immunoglobulin is a heat stable immunoglobulin that selectively binds to a mammalian $Fc_\epsilon R$ molecule.

5. The invention of Claim 1 or 3, wherein said invention detects a biologically active, allergen-specific immunoglobulin not detectable by a process using anti-IgE antibodies.

6. The invention of Claim 1-3, wherein compositions isolated from at least about 5% of a population of mammals comprise biologically active, allergen-specific immunoglobulins not detectable by a process using anti-IgE antibodies.

7. The invention of Claim 1-3, wherein compositions isolated from about 10% of a population of mammals comprise biologically active, allergen-specific immunoglobulins not detectable by a process using anti-IgE antibodies.

8. The invention of Claim 1-4, wherein said immunoglobulin, upon interaction with said allergen and a cell that naturally has a $Fc_{\epsilon}R$, is capable of activation of and mediator release from said cell.

9. The invention of Claim 1-4, wherein said $Fc_{\epsilon}R$ molecule comprises at least a portion of a $Fc_{\epsilon}R$ alpha chain that is capable of binding to IgE.

10. The invention of Claim 1-4, wherein said $Fc_{\epsilon}R$ molecule is selected from the group consisting of a human $Fc_{\epsilon}R$ molecule and a companion animal $Fc_{\epsilon}R$ molecule.

11. The invention of Claim 1-4, wherein said $Fc_{\epsilon}R$ molecule is selected from the group consisting of a human $Fc_{\epsilon}R$ molecule, a feline $Fc_{\epsilon}R$ molecule, a canine $Fc_{\epsilon}R$ molecule, and an equine $Fc_{\epsilon}R$ molecule.

12. The invention of Claim 1-4, wherein said immunoglobulin is IgE.

13. The invention of Claim 1-4, wherein said immunoglobulin is an IgE-like immunoglobulin.

14. The invention of Claim 1-3, wherein said immunoglobulin is a heat stable immunoglobulin.

15. The invention of Claim 1-4, wherein said immunoglobulin is selected from the group consisting of a human immunoglobulin and a companion animal immunoglobulin.

16. The invention of Claim 1-4, wherein said immunoglobulin is selected from the group consisting of a human immunoglobulin, a feline immunoglobulin, a canine immunoglobulin, and an equine immunoglobulin.

17. The invention of Claim 1 or 2, wherein said putative immunoglobulin-containing composition comprises a composition selected from the group consisting of blood, serum, plasma, urine, tears, aqueous humor, central nervous system fluid, saliva, lymph, nasal secretions, milk and feces.

18. The invention of Claim 1-3, wherein said $Fc_\epsilon R$ molecule is conjugated to a detectable marker.

19. The invention of Claim 1-3, wherein said step of detecting of Claim 1 or 2 or said detection means of Claim 3 comprises performing an assay selected from the group consisting of an enzyme-linked immunoassay, a radioimmunoassay, an immunoprecipitation assay, a fluorescence immunoassay, a chemiluminescent assay, an immunodot assay, an immunoblot assay, a lateral flow assay, an agglutination assay and a particulate-based assay.

20. The invention of Claim 1 or 2 further comprising the step selected from the group consisting of: binding said $Fc_\epsilon R$ molecule to a substrate prior to performing step (a) to form a $Fc_\epsilon R$ molecule-coated substrate; binding said allergen to a substrate prior to performing step (a) to form an allergen-coated substrate; and binding said putative immunoglobulin-containing composition to a substrate prior to performing step (a) to form a putative immunoglobulin-containing composition-coated substrate, wherein said substrate is selected from the group consisting of a non-coated substrate, a $Fc_\epsilon R$ molecule-coated substrate, and an allergen-coated substrate.

21. The invention of Claim 1-3, wherein said method of Claim 1 or 2 or said detection means of Claim 3 is performed in solution.

22. The invention of Claim 1-3, wherein said method of Claim 1 or 2 or said detection means of Claim 3 does not require a washing step.

23. The invention of Claim 1-3, wherein said composition does not require dilution.

24. The invention of Claim 1-3, wherein said method of Claim 1 or 2 or said detection means of Claim 3 is selected from the group consisting of a single-step assay and a dual-step assay.

25. The kit of Claim 3, wherein said detection means detects said $Fc_\epsilon R$ molecule.

26. The kit of Claim 3, wherein said allergen is immobilized on a substrate or wherein said $Fc_\epsilon R$ molecule is immobilized on a substrate.

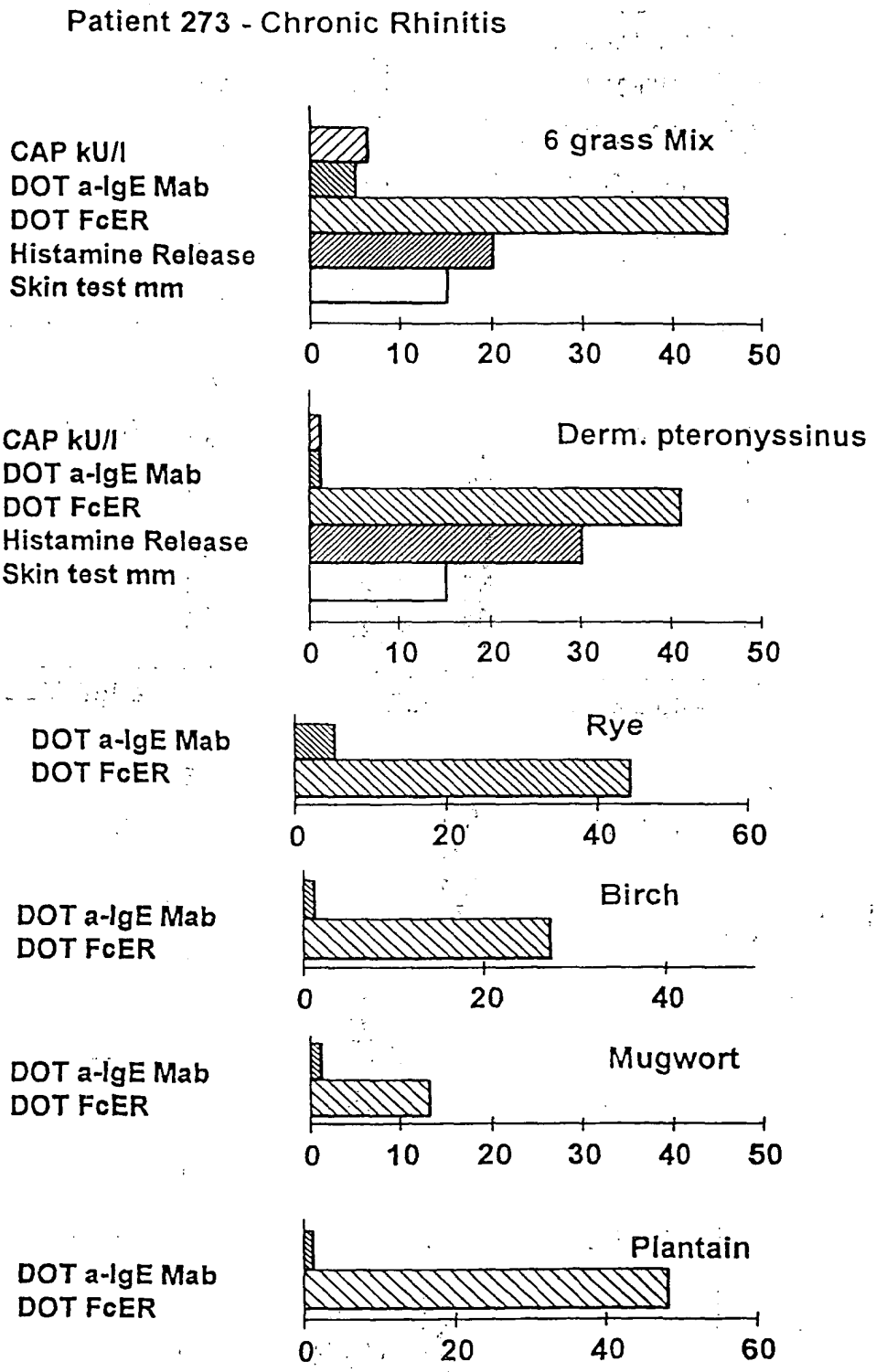
Patient 273 - Chronic Rhinitis
CAP kU/l
DOT a-IgE Mab
DOT FcER
Histamine Release
Skin test mm
6 grass Mix
0 10 20 30 40 50
CAP kU/l
DOT a-IgE Mab
DOT FcER
Histamine Release
Skin test mm
Derm. pteronyssinus
0 10 20 30 40 50
DOT a-IgE Mab
DOT FcER
Rye
0 20 40 60
DOT a-IgE Mab
DOT FcER
Birch
0 20 40
DOT a-IgE Mab
DOT FcER
Mugwort
0 10 20 30 40 50
DOT a-IgE Mab
DOT FcER
Plantain
0 20 40 60

FIG. 1

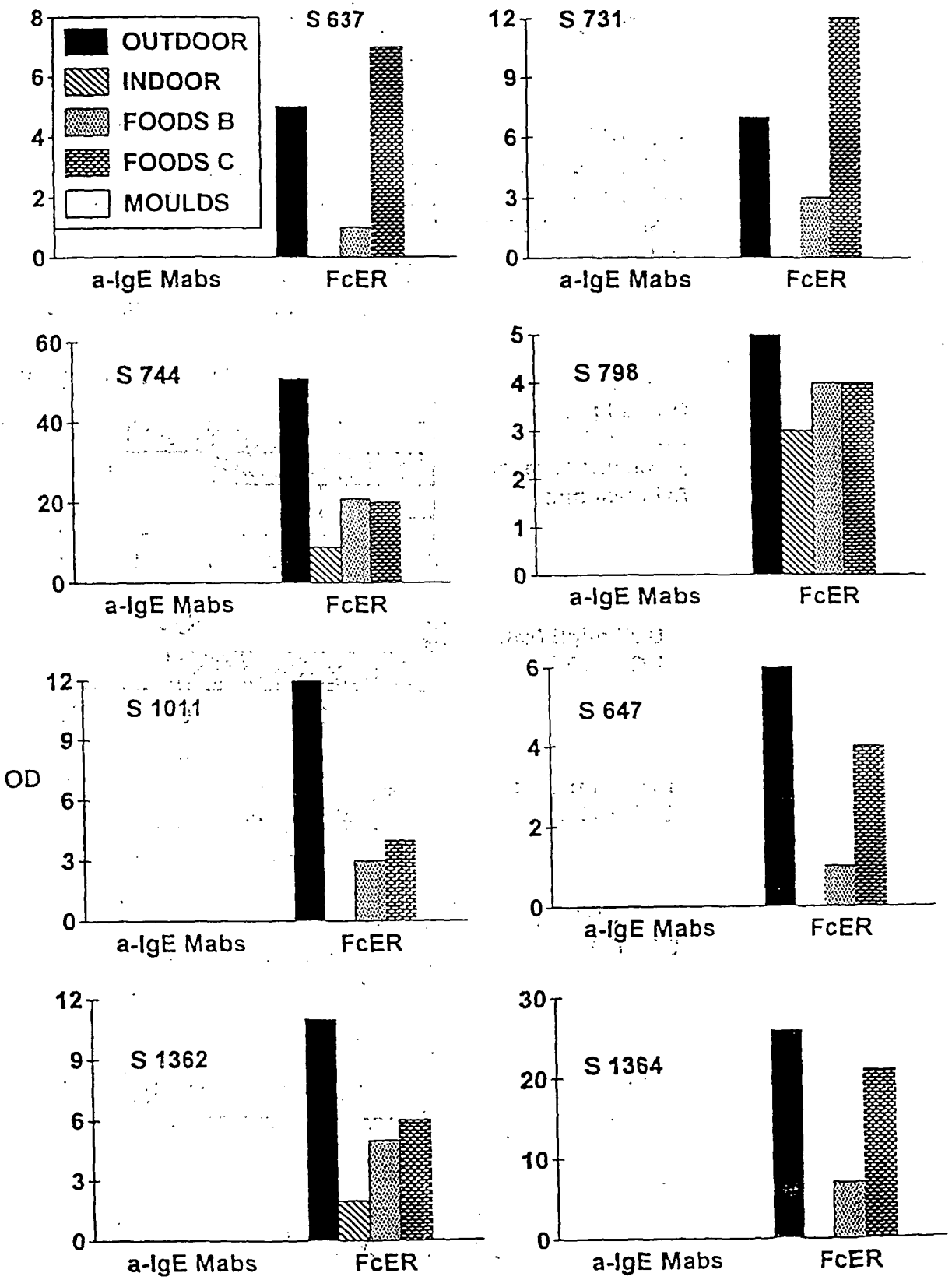
OUTDOOR
INDOOR
FOODS B
FOODS C
MOULDS
S 637
S 731
S 744
S 798
S 1011
S 647
S 1362
S 1364
OD
a-IgE Mabs
FcER

FIG. 2

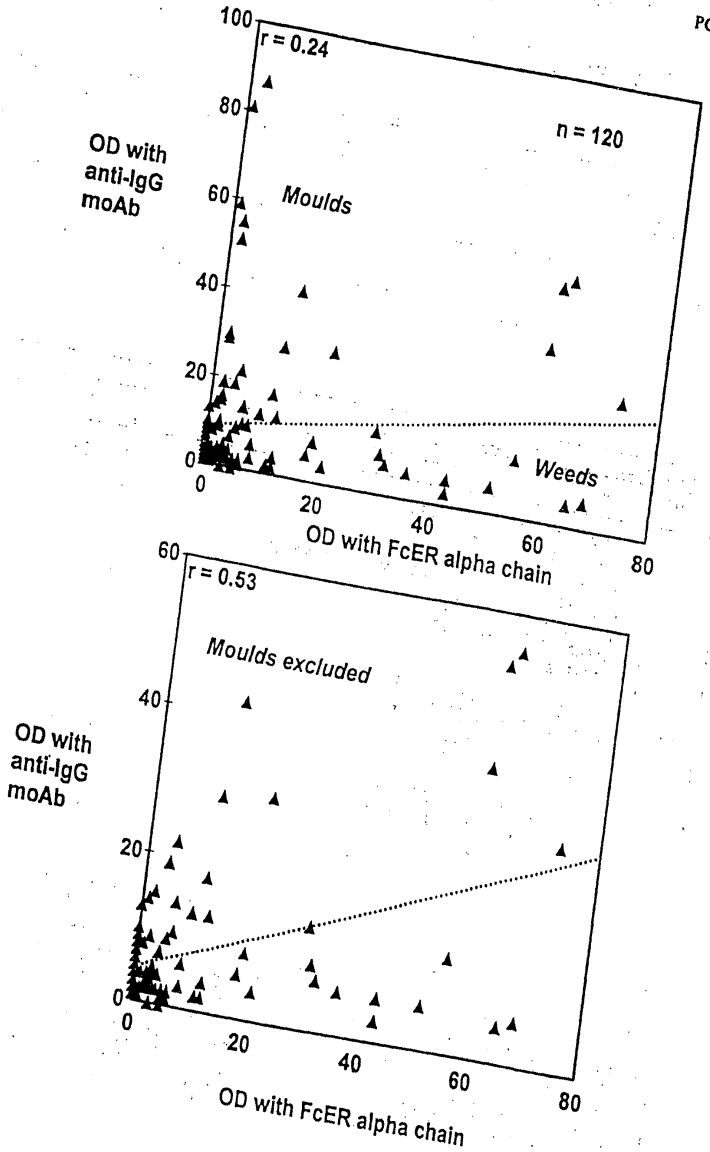
r = 0.24
n = 120
Moulds
Weeds
OD with anti-IgG moAb
OD with FcER alpha chain
r = 0.53
Moulds excluded
OD with anti-IgG moAb
OD with FcER alpha chain

FIG. 3

INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/US 99/07530

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/68 C07K16/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 096, no. 008, 30 August 1996 (1996-08-30) & JP 08 101194 A (TONEN CORP;RA TOMOYASU), 16 April 1996 (1996-04-16) cited in the application abstract	1-4
P,X	WO 98 23964 A (HESKA CORP) 4 June 1998 (1998-06-04) cited in the application the whole document	1-4
P,X	WO 98 45707 A (HESKA CORP) 15 October 1998 (1998-10-15) the whole document	1-4

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 July 1999	06/08/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreno, C

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/US 99/07530

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO 97 20859 A (IDEXX LAB INC) 12 June 1997 (1997-06-12) abstract ---	1-4
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INTERNATIONAL SEARCH REPORT

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